

**REMARKS**

Upon entry of this response and amendment, claims 1-22 are pending. The claims and specification have been amended in the anticipation that the amendments will place the application in condition for allowance, and to better clarify the subject matter to be protected. Applicant respectfully submits that the amendments are based on the specification as originally filed, and therefore do not add any new matter within the meaning of 35 U.S.C. §132. The amendments will be commented on in further detail below.

Applicants respectfully submit that all pending claims are patentable over the cited prior art for the following reasons, and that the application is now in condition for allowance. Early acknowledgment of this is earnestly solicited. The objections and rejections will now be addressed individually.

**1. Objection to the Specification**

The specification has been objected to for the following reasons:

The specification is objected to for numerous misspellings and improper use of abbreviations (sic). For example:  
Page 9, line 9 and elsewhere: histidin should be histidine  
Page 11, line 1 and elsewhere: fluoresceine should be fluorescein  
Page 17, line 32 and elsewhere: bidistilled should be bidistilled  
Page 18, line 14 and elsewhere: glutathion should be glutathione

Page 22, line 4 and elsewhere: splicevariants should be splice variants  
NTA and SOD should be defined at their first appearance and then used throughout.

### **RESPONSE**

Applicant respectfully requests reconsideration and withdrawal of this objection.

Applicant has amended the specification to correct the spelling of the above words throughout the specification. Applicant thanks the Examiner for the helpful suggestions for overcoming these objections. With respect to NTA and SOD, Applicant respectfully points out that NTA is clearly defined on page 9, and SOD on page 5, of the specification, respectively.

Accordingly, based on the enclosed amendments to the specification, Applicant has obviated these objections. Applicant, therefore, requests reconsideration and withdrawal of the objections.

### **2. Objection to the Claims**

Claims 1-22 stand objected to as being replete with terms, phrases, and claim construction that lacks clarity. The claims are objected to for the following reasons:

In claims 1, 14 and 18, and claims dependent thereon, the word enzymatical is not proper English, enzymatic is the correct word.

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claims 1, 14 and 18 to replace "enzymatical" with "enzymatic." In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

In Claims 1 and 3 and claims dependent thereon, "...forming of a complex..." has less clarity than "...formation of a complex...". Appropriate correction is required.

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claims 1 and 3 to replace "forming" with "formation." In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

In Claim 1 and claims dependent thereon, the phrase "...under incubation with..." lacks clarity. Clarity would be provided by reciting "...in the presence of...".

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claim 1 replace "under incubation with" with "in the presence of." In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

The abbreviation NTA should be defined at the first appearance of nitrilotriactat, in claim 11, and used thereon.

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claim 11 to define "NTA" and the other claims to use the newly defined abbreviation. In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

Claim 13 is objected to for lack of clarity. The phrase "...in the complex formation step additionally calmodulin and/or calcium is added" is confusing and poor grammar. Clarity would be provided by using the phrase: "...in the complex formation step, clamodulin and/or calcium are present."

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claim 13 per the Examiner's suggestion. In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

Claim 18 is objected to for lack of clarity. Clarity would be provided if Claim 18 was constructed to simply state that the influences of the potential modulator on the complex formation and on complex activity are analyzed separately.

(Also)

Claim 18 is objected to; seperately should be corrected to separately.

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claim 18 in accordance with the Examiner's suggestions. In doing so, Applicant has removed the basis for these objections, and respectfully requests reconsideration and withdrawal thereof.

Claim 17 is objected to; fluoresceine should be corrected to fluorescein.

**RESPONSE**

Applicant thanks the Examiner for the helpful suggestion. Applicant has amended claims 1, 14 and 18 to replace "enzymatical" with "enzymatic." In doing so, Applicant has removed the basis for this objection, and respectfully requests reconsideration and withdrawal thereof.

**3. Rejection of Claims 1-22 Under  
35 U.S.C. §112, Second Paragraph**

Claims 1-22 stand rejected under 35 U.S.C. §112, second paragraph as being indefinite. The Office Action provides the following as a basis for the rejection:

Claims 1 and 18 recites the broad recitation "activity" and the claim also recites "especially the enzymatic activity" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claims 1 and 18 to remove the phrase

"especially the enzymatic activity," thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 5 recites the broad recitation "labels", and the claim also recites "especially fluorescent labels" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 5 to remove the phrase "especially fluorescent labels" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 6 recites the broad recitation "labels", and the claim also recites "especially fluorescent marker" which is the narrower statement of the range/limitation.

Claim 6 recites the broad recitation "labels", and the claim also recites "preferably the labels are enhanced green fluorescent protein" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 6 to remove the narrower recitations as listed above. In doing so, Applicant has removed the basis for this rejections. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 7 recites the broad recitation "fluorescent proteins", and the claim also recites "particularly as fusion proteins together with green fluorescent proteins" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 7 to remove the phrase "particularly as fusion proteins together with enhanced green fluorescent protein" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 9 recites the broad recitation "cells", and the claim also recites "especially in eukaryotic cells" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 9 to remove the phrase "especially in eukaryotic cells" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 10 recites the broad recitation "cells", and the claim also recites "especially in prokaryotic cells" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 10 to remove the phrase

"especially in prokaryotic cells" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 11 recites the broad recitation "affinity chromatography", and the claim also recites "especially by ferro-nitrilotriacetat-metal affinity chromatography" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 11 to remove the inconsistency between the broad and narrow ranges contained therein. In doing so, Applicant has removed the basis for this rejection. Therefore, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 12 recites the broad recitation "affinity chromatography", and the claim also recites "especially by copper/zinc-nitrilotriacetat-metal affinity chromatography" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 12 to remove the inconsistency between the broad and narrow ranges contained therein. In doing so, Applicant has removed the basis for this rejection. Therefore, Applicant respectfully requests reconsideration and withdrawal of the rejection.



Claim 15 recites the broad recitation "a label", and the claim also recites "especially a fluorescent label" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 15 to remove the phrase "especially a fluorescent label" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 16 recites the broad recitation "peptide", and the claim also recites "especially a peptide characterized by the amino acid sequence..." which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 16 to remove the phrase "especially a peptide" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 17 recites the broad recitation "a residue", and the claim also recites "especially a serine residue" which is the narrower statement of the range/limitation.

**RESPONSE**

Applicant has amended claim 17 to remove the phrase "especially in serine residue" thus removing the narrower statement

of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 19 recites the broad recitation "method", and the claim also recites especially according to claim 1" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 19 to remove the phrase "especially according to claim 1" thus removing the narrower statement of the range or limitation and making claim 19 an independent claim. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 20 recites the broad recitation "tag", and the claim also recites "especially a histidine tag" which is the narrower statement of the range/limitation.

#### **RESPONSE**

Applicant has amended claim 20 to remove the phrase "especially a histidine tag" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

Claim 22 recites the broad recitation "a solid matrix",

and the claim also recites "especially a Ni-NTA, Fe-NTA and/or CuZn-NTA matrix" which is the narrower statement of the range/limitation.

#### RESPONSE

Applicant has amended claim 22 to remove the phrase "especially a Ni-NTA, Fe-NTA and/or CuZn-NTA matrix" thus removing the narrower statement of the range or limitation. Accordingly, Applicant has also removed the basis for this rejection. Applicant respectfully requests reconsideration and withdrawal of the rejection.

The term "activity" in Claim 1 is an undefined term that renders Claim 1 indefinite. The specification does not provide any examples, other than measure calcineurin enzymatic activity, for ascertaining the "activity" of the complex and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention.

#### RESPONSE

Applicant has amended claim 1 to recite that the "activity" is now defined as "enzymatic activity." By making this amendment, Applicant has clarified that the activity referred to in the claim is the calcineurin enzymatic activity.

Applicant respectfully submits that the invention as claimed is not a method for treatment based on the activity of calcineurin. Instead, the invention is that of **an assay for determining the activity of a calcineurin/SOD complex, possibly in the presence of a potential modulator.** For example, it is known that calcineurin

activity produces phosphorylation of serine amino acid. This activity by the calcineurin/SOD complex would be a measurement taken to determine the calcineurin activity. The measurement is compared to base-line activity based on the calcineurin and SOD used in the complex formation.

Thus, by making the above amendment, Applicant has clarified the subject matter. Applicant, therefore, submits that the basis for this rejection has been removed, and respectfully requests reconsideration and withdrawal of the rejection.

In Claim 10, it is unclear whether calcineurin and SOD are coexpressed in the same cell or are expressed in separate cells. It is also unclear whether the complex being analyzed is being formed in vitro. An interpretation of Claim 10 is that calcineurin and SOD are coexpressed in the same cells, that complex formation occurs in the intact cell, and only the uncomplexed calcineurin or SOD are isolated. Alternatively, Claim 10 could be interpreted as a method whereby calcineurin and SOD are expressed in different cells, that free calcineurin or SOD are isolated from the respective cells, and that complex formation occurs in vitro.

#### **RESPONSE**

Applicant respectfully traverses this rejection and request reconsideration and withdrawal thereof.

Applicant respectfully submits that the present invention, as claimed in the application, is directed to an assay for determining calcineurin activity. The activity is monitored in the presence of a potential modulator, and the influence of such modulator is determined by directly monitoring the complex formation of a calcineurin/SOD complex and/or the enzymatic activity of the

calcineurin.

According to claims 10-12, the claims under rejection here, the calcineurin and/or SOD are expressed in cells, isolated and/or purified before the complex is formed. Applicant respectfully submits that it is irrelevant whether the calcineurin and SOD are expressed in the same or different cells because the calcineurin and SOD are isolated and/or purified prior to being mixed together to form the complex. According to claim 10 (and the specification), the isolation occurs **before** the complex formation. In other words, the calcineurin and SOD are expressed in cells, then isolated and/or purified before being mixed together for the formation of the complex. The end result is the same if the calcineurin and SOD are expressed in the same or different cells, because they are isolated after such expression.

Accordingly, Applicant respectfully submits that claim 10-12 are definite regarding the expression of the calcineurin and SOD in the cells, as is required by claim 10. Applicant, therefore, respectfully requests reconsideration and withdrawal of this rejection.

Claim 13 is further rejected under 35 U.S.C. 112, second paragraph....It is unclear when (in claim 13), during complex formation, calmodulin and/or calcium are added.

#### **RESPONSE**

Applicant respectfully traverses this rejection and request

reconsideration and withdrawal thereof.

Applicant respectfully submits that the present invention is directed to an assay for determining calcineurin activity. The activity is monitored in the presence of a potential modulator, and the influence of such modulator is determined by directly monitoring the complex formation of a calcineurin/SOD complex and/or the enzymatic activity of the calcineurin. In other words, the invention involves mixing calcineurin and SOD to form a complex, then monitoring the effects of potential modulators on that complex by monitoring the complex formation and/or the enzymatic activity of (the calcineurin in) the complex.

Applicant respectfully submits that it is irrelevant **when** the calcium and/or calmodulin are added because the important aspects of the invention involve the complex formation and the addition of calcium and/or calmodulin may be one variable evaluated by the assay of the present inventive subject matter. One of ordinary skill in the art will recognize that it is irrelevant when the calcium and/or calmodulin are added for practicing the presently claimed inventive subject matter. Thus, Applicant respectfully submits that the claim 13 is definite, and request reconsideration and withdrawal of the rejection of the claim as being indefinite for the reasons given in the Office Action.

Claim 19 recites the method according to Claim 1. However, the methods of Claim 19 fail to further limit the methods of Claim 1 as, the methods of Claim 19 are distinct from the methods of Claim 1.

**RESPONSE**

Applicant has amended claim 19 to remove the dependency of the claim from claim 1, thereby making claim 19 an independent claim. By making the amendment, Applicant has removed the basis for this rejection. Applicant, therefore, respectfully requests reconsideration and withdrawal of the rejection.

**4. Rejection of Claims 1-22 Under  
35 U.S.C. §112, First Paragraph**

Claims 1-22 stand rejected under 35 U.S.C. §112, first paragraph as being based on a non-enabling specification for the reasons set forth in the Office Action.

**RESPONSE**

Applicant respectfully traverses this rejection and respectfully requests reconsideration and withdrawal thereof.

The Examiner argues that this rejection should be made because the claims allegedly do not enable the methods of using any form of calcineurin or any form of SOD. However, Applicant respectfully disagrees with this assertion, and requests reconsideration and withdrawal of the rejection.

Applicant respectfully submits that calcineurin and SOD from any source **show a certain degree of sequence identity** and, more importantly, **show the same functional characteristics**. Since the inventive subject matter as claimed is based on the functional characteristics of the enzymes, the claimed subject matter works

with **all** calcineurin and SOD from **any** source showing the functional characteristics of these enzymes. One of ordinary skill in the art will recognize this.

Furthermore, Applicant respectfully submits that the present inventive subject matter is directed to an assay to determine calcineurin activity. It is irrelevant from where the calcineurin comes **since it is the activity of that particular calcineurin which is under evaluation.** In other words, because a particular calineurin is being used, it is that calcineurin which is being monitored and evaluated. Likewise, the method is limited to those calcineurins and SOD's **which form a complex.** Thus, it is also irrelevant from where the SOD comes **so long as it forms a complex with the calcineurin.** One of ordinary skill in the art will recognize that if the calcineurin and SOD are incompatible, i.e., do **not** form a complex, then the calcineurin and SOD fall outside the scope of the present claims. The only limitation of the calcineurin and SOD to be used is that **they must form the complex.**

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claim 1-22 as being based on a non-enabling specification.

**5. Rejection of Claims 1-5, 9, 13-16, 18, 19 and 21  
Under 35 U.S.C. §102(b)**

Claims 1-5, 9, 13-16, 18, 19 and 21 stand rejected under 35 U.S.C. §102(b) as being anticipated by Wang et al. (1996).



**RESPONSE**

Applicants respectfully traverse this rejection and request reconsideration and withdrawal thereof.

To establish an anticipation rejection, every claimed element must be found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros. V. Union Oil Co. of California*, 814 F2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987); See also, MPEP § 2131. Applicants respectfully submit that Wang et al. do not teach every claimed element within the independent claims, and therefore also in the dependent claims, thereby failing to anticipate the claims.

As amended, claim 1 is drawn to a method for screening of modulators of calcineurin enzymatic activity. The method is characterized in that an interaction between calcineurin and superoxide dismutase is monitored. The method comprises the following steps: formation of a complex comprising at least calcineurin and superoxide dismutase in the presence of at least one potential modulator, and detecting the influence of the potential modulator by directly monitoring the complex formation and/or by monitoring the enzymatic activity.

Likewise, independent claim 19 is drawn to a method for screening of modulators of calcineurin activity comprising: a) determining the interaction of a potential modulator with either calcineurin or superoxide dismutase as a partner, b) taking a

potential modulator showing interaction with calcineurin or superoxide dismutase according to step a), c) determining the interaction of said modulator taken in step b), with the other partner, namely calcineurin or superoxide dismutase, respectively, and d) identifying the potential modulator showing interaction also according to step c).

The claimed subject matter results in a direct protein-protein interaction of between the SOD (CuZnSOD) and calcineurin. This results in the formation of the calcineurin/SOD complex. Applicant has shown this direct interaction (i.e. the formation of the complex) in a qualitative and quantitative manner using the pull-down assay and Laser Correlation Spectroscopy. Furthermore, Applicant has been able to show that the hydrodynamic radius and binding of mutated CuZnSOD, which is relevant in clinical aspects regarding familial Amyotrophic Lateral Sclerosis, is qualitatively altered on the molecular level. This phenomena directly results in an altered protective behavior towards calcineurin activity. Therefore, Applicant has shown that CuZnSOD protection of calcineurin is not an indirect enzymatic effect, but is linked to a direct protein-protein interaction between the calcineurin and the two metallo enzymes. This suggests a direct electron transfer between the CuZnSOD Cu/Zn metallo center and the Fe/Zn metallo center of calcineurin, thus resulting in the formation of the complex.

Applicant respectfully submits that Wang et al. fail to

disclose the invention as captured in all of the claimed elements detailed above. In particular, Applicant respectfully submits that Wang et al. do not show the direct interaction of the calcineurin and SOD as is required by the claims of the present application.

Wang et al. show the co-purification of calcineurin and SOD after gel permeation chromatography of crude brain homogenate. However, this does not show the interaction of calcineurin and SOD since the crude fractions still contain more than 1,000 different proteins which may interact with the chromatographic matrix or other proteins in an unspecific manner. The crude fraction may also be part of proteins enclosed by phospholipids or other miscelles. Further, the crude fraction may exhibit similar gel permeation coefficients by forming oligomers. For example, four CuZnSOD subunits (at 16,000 D = 64,000 Daltons) have a similar molecular weight as calcineurin A (60,000 Daltons) and therefore would penetrate a gel chromatography column at the same time as the calcineurin A. Thus, the findings of Wang et al. do not scientifically prove interaction between calcineurin and SOD, as is required by the present claims.

Furthermore, Applicant respectfully submits that Wang et al. do not prove the protein-protein interaction in its discussion of the protective effect of CuZnSOD in a calcineurin/CuZnSOD assay mixture. Wang et al. allegedly show that CuZnSOD protects calcineurin against inactivation. They discuss that super oxide radicals or other reactive oxygen intermediates (ROI) directly act

on the sensitive mineral center of calcineurin. This suggests that the protection of calcineurin by CuZnSOD is the result of an indirect effect of CuZnSOD enzymatic activity. However, this means that the CuZnSOD enzyme reduces super oxide radicals and thereby also other ROI's **by simply applying its super oxide dismutase activity**, i.e.,  $2 O^{2-} + 2 H^+ \rightarrow H_2O_2 + O_2$ . Applicant respectfully submits, though, **that such a mechanism does not need a direct protein interaction between calcineurin and CuZnSOD**. Thus, there is no proof of the interaction of the proteins just because CuZnSOD has a positive effect on a calcineurin/CuZnSOD assay mixture. It can be seen, therefore, that Wang et al. do **not** disclose any scientific basis for the formation of the complex, as is required in the instant claims.

In summary, Applicant has been the first to show a direct interaction between the two proteins. Since Wang et al. do not show this interaction between SOD and calcineurin, especially the formation of the complex, their disclosure fails to anticipate the subject matter as claimed.

Accordingly, Applicant respectfully submits that Wang et al. fail to disclose every claimed element of independent claims 1 and 19, and therefore the dependent claims, and thus does not anticipate the claims under 35 U.S.C. 102(b). Applicant respectfully requests reconsideration and withdrawal of the rejection of the claims as being anticipated by the reference.

**6. Rejection of the Claims  
Under 35 U.S.C. 103(a)**

Claims 5-8 stand rejected under 35 U.S.C. 103(a) as being obvious over Wang et al. (1996) in view of Brown et al. (1997); claim 9 stands rejected as being obvious over Wang et al. (1996) in view of Woodrow et al. (1997); claims 10-12 stand rejected as being obvious over Wang et al. (1996) in view of Lau et al. (1996) or Robbins et al. (1993) and further in view of Aramburu et al. (1998); and claims 5, 15 and 17 stand rejected as being obvious over Wang et al. (1996) in view of the specification. The reasons for the rejections are set forth in the Office Action. Since all of the obviousness rejections rely on Wang et al. as the primary reference, the rejections will be dealt with together.

**RESPONSE**

Applicants respectfully traverse this rejection and request reconsideration and withdrawal thereof.

The references of record as listed above, do not teach or suggest applicant's inventive subject matter as a whole, as recited in the claims. Further, there is no teaching or suggestion in these references which would lead the ordinary skilled artisan to modify the references to derive the subject matter as defined in the amended claims.

The U.S. Supreme Court in *Graham v. John Deere Co.*, 148 U.S.P.Q. 459 (1966) held that non-obviousness was determined under

§ 103 by (1) determining the scope and content of the prior art; (2) ascertaining the differences between the prior art and the claims at issue; (3) resolving the level of ordinary skill in the art; and, (4) inquiring as to any objective evidence of nonobviousness.

To establish a *prima facie* case of obviousness, the Examiner must establish: (1) that some suggestion or motivation to modify the references exists; (2) a reasonable expectation of success; and (3) that the prior art references teach or suggest all the claim limitations. Amgen, Inc. v. Chugai Pharm. Co., 18 USPQ2d 1016, 1023 (Fed. Cir. 1991); In re Fine, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988); In re Wilson, 165 USPQ 494, 496 (C.C.P.A. 1970).

A *prima facie* case of obviousness must also include a showing of the reasons why it would be obvious to modify the references to produce the present invention. See Ex parte Clapp, 277 USPQ 972, 973 (Bd. Pat. App. & Inter. 1985). The Examiner bears the initial burden to provide some convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings. Id. at 974.

Claims 1 and 19 are the independent claims, with the remaining claims depending therefrom. Thus, if claims 1 and 19 are not obvious over the prior art references, then neither are the dependent claims. As amended, claim 1 is drawn to a method for screening of modulators of calcineurin enzymatic activity. The method is characterized in that an interaction between calcineurin

and superoxide dismutase is monitored. The method comprises the following steps: formation of a complex comprising at least calcineurin and superoxide dismutase in the presence of at least one potential modulator, and detecting the influence of the potential modulator by directly monitoring the complex formation and/or by monitoring the enzymatic activity.

Likewise, independent claim 19 is drawn to a method for screening of modulators of calcineurin activity comprising: a) determining the interaction of a potential modulator with either calcineurin or superoxide dismutase as a partner, b) taking a potential modulator showing interaction with calcineurin or superoxide dismutase according to step a), c) determining the interaction of said modulator taken in step b), with the other partner, namely calcineurin or superoxide dismutase, respectively, and d) identifying the potential modulator showing interaction also according to step c).

In addition, the dependent claims also contain the above limitations as is detailed above.

As is discussed above with respect to the anticipation rejection, the claimed subject matter results **in a direct protein-protein interaction of between the SOD (CuZnSOD) and calcineurin.** This further results in the formation of the calcineurin/SOD complex. Applicant has shown **this direct interaction** (i.e. the formation of the complex) in a qualitative and quantitative manner using the pull-down assay and Laser Correlation Spectroscopy.

Furthermore, Applicant has been able to show that the hydrodynamic radius and binding of mutated CuZnSOD, which is relevant in clinical aspects regarding familial Amyotrophic Lateral Sclerosis, is qualitatively altered on the molecular level. This phenomena directly results in an altered protective behavior towards calcineurin activity. Therefore, **Applicant has shown that CuZnSOD protection of calcineurin is not an indirect enzymatic effect, but is linked to a direct protein-protein interaction between the calcineurin and the two metallo enzymes. This suggests a direct electron transfer between the CuZnSOD Cu/Zn metallo center and the Fe/Zn metallo center of calcineurin, thus resulting in the formation of the complex.**

In the section above, Applicant has shown that Wang et al. do not disclose the direct interaction of the calcineurin and SOD as is required by the claims of the present application. Wang et al. show the co-purification of calcineurin and SOD after gel permeation chromatography of crude brain homogenate. However, **this does not show the interaction of calcineurin and SOD** since the crude fractions still contain more than 1,000 different proteins which may interact with the chromatographic matrix or other proteins in an unspecific manner. The crude fraction may also be part of proteins enclosed by phospholipids or other miscelles. Further, the crude fraction may exhibit similar gel permeation coefficients by forming oligomers. For example, four CuZnSOD subunits (at 16,000 D = 64,000 Daltons) have a similar molecular



weight as calcineurin A (60,000 Daltons) and therefore would penetrate a gel chromatography column at the same time as the calcineurin A. Thus, **the findings of Wang et al. do not scientifically prove interaction between calcineurin and SOD, as is required by the present claims.**

Furthermore, Applicant respectfully submits that Wang et al. **do not prove the protein-protein interaction in their discussion of the protective effect of CuZnSOD in a calcineurin/CuZnSOD assay mixture.** Wang et al. allegedly show that CuZnSOD protects calcineurin against inactivation. They discuss that super oxide radicals or other reactive oxygen intermediates (ROI) directly act on the sensitive mineral center of calcineurin. This suggests that the protection of calcineurin by CuZnSOD is the result of an indirect effect of CuZnSOD enzymatic activity. However, this means that the CuZnSOD enzyme reduces super oxide radicals and thereby also other ROI's **by simply applying its super oxide dismutase activity**, i.e.,  $2 O^{2-} + 2 H^+ \rightarrow H_2O_2 + O_2$ . Applicant respectfully submits, though, **that such a mechanism does not need a direct protein interaction between calcineurin and CuZnSOD.** Thus, there is no proof of the interaction of the proteins just because CuZnSOD has a positive effect on a calcineurin/CuZnSOD assay mixture. It can be seen, therefore, that Wang et al. do **not** disclose any scientific basis for the formation of the complex, as is required in the instant claims.

Thus, since Wang et al. fail to disclose the limitations of

the claims, Applicant respectfully submits that claims 5, 15 and 17 are not obvious over Wang et al. in view of the specification, since the quoted section of the specification does not remedy the deficiencies of Wang et al. In particular, the cited section of the specification does not teach the formation of the complex, as is required by the independent claims, and therefore claims 5, 15 and 17, which depend from claim 1. Accordingly, claims 5, 15 and 17 are not obvious over Wang et al.

Turning now to the remaining claims, Applicant respectfully submits that the other references of record relied upon by the Examiner in making the rejections also do not remedy the deficiencies of Wang et al. The Examiner asserts that Brown et al. teaches the use of fluorescence-labeled proteins for assaying protein-protein interactions. While this may be true, Applicant points out that Brown et al. do **not discuss** the particular protein-protein interaction as claimed in the present application. Thus, Applicant submits that Brown et al. does not remedy the deficiency of Wang et al. with respect to the independent claims, and therefore Brown et al. does not remedy the deficiency with respect to claims 5-8 as put forth by the Examiner. In particular, Brown et al. **are also silent with respect to the formation of the calcineurin/SOD complex**, as is required by the claims.

Further, since neither reference discloses the formation of the above complex, Applicant respectfully submits that the combination of references would still lack the important limitation

of the format of the complex. Such a deficiency in the combination results in the Examiner failing to meet the burden of proving a *prima facie* case of obviousness. Applicant, therefore, submits that claims 5-8 are not obvious over the prior art, and requests reconsideration and withdrawal of the rejection.

Turning now to Woodrow et al., Applicant respectfully submits that Woodrow et al. also do not cure the deficiency of Wang et al. The Examiner relies on Woodrow et al. in the rejection of claim 9, however, as with Brown et al. discussed above, Applicant submits that Woodrow et al. do not disclose the formation of the calcineurin/SOD complex as is required by the claims.

Woodrow et al. disclose the regulation of calcineurin in cells upon cotransfection with a NFAT reporter construct. However, Woodrow et al. **are silent with respect to the formation of the calcineurin/SOD complex formation**, which is required in claim 1, and therefore also in claim 9 which depends from claim 1. Since neither Wang et al. nor Woodrow et al. disclose the formation of the protein complex, as detailed above, Applicant respectfully submits that the combination of references would still be deficient in attempting to achieve the claimed invention. Such a deficiency in the combination results in the Examiner failing to meet the burden of proving a *prima facie* case of obviousness. Applicant, therefore, submits that claim 9 is not obvious over the prior art, and requests reconsideration and withdrawal of the rejection.

The Examiner relies on Lau et al., Robbins et al., and

Aramburu et al., to try to remedy the deficiency of Wang et al. with respect to claims 10-12. However, Applicant respectfully submits that the three references fail to remedy Wang et al. with respect to claim 1, from which claims 10-12 depend, and therefore do not render obvious claims 10-12.

Lau et al. teach the use of tagged fusion proteins to test the interaction of the receptor with SAP102. The fused proteins are tagged with histidine. However, Applicant respectfully submits that Lau et al. **are silent with respect to the formation of the calcineurin/SOD complex formation**, which is required in claim 1, and therefore also in claims 10-12 which depend from claim 1. Since neither Wang et al. nor Lau et al. disclose the formation of the protein complex, as detailed above, Applicant respectfully submits that the combination of references would still be deficient in attempting to achieve the claimed invention. The deficiency in the combination results in the Examiner failing to meet the burden of proving a *prima facie* case of obviousness. Applicant, therefore, submits that claims 10-12 are not obvious over these prior art references, and requests reconsideration and withdrawal of the rejection.

The Examiner also relies on Robbins et al. in making this rejection. Applicant submits, however, the Robbins et al. are also silent with respect to the deficiency as found in Wang et al. and Lau et al. In particular, Robbins et al. teach the purification of recombinant histidine-tagged ERK2 prior to analysis for the

regulation of kinase activity in vitro. Robbins et al. **do not** teach the formation of the calcineurin/SOD complex, as is required by claim 1, and therefore by claims 10-12 which depend therefrom. Much like Wang et al. and Lau et al. above, the combination of Wang et al. and Robbins et al. would be deficient in its attempt to attain the presently claimed subject matter. Such a combination would lack the requirement **of the formation of the complex between the calcineurin and the SOD**. This deficiency in the combination results in the Examiner **failing to meet the burden of proving a prima facie case of obviousness**. Applicant, therefore, submits that claims 10-12 are not obvious over the combination of references discussed thus far, and requests reconsideration and withdrawal of the rejection.

The third reference that the Examiner relies on in rejecting claims 10-12 as being obvious is the Aramburu et al. reference. For the same reasons as espoused above, Applicant respectfully submits that the claims are not obvious over Aramburu et al. in combination with any of the previously discussed references. In particular, Applicant has shown that none of Wang et al., Lau et al., and Robbins et al. has taught the limitation of the formation of the calcineurin/SOD complex. Likewise, this limitation is **not** shown in the Aramburu et al. reference.

Aramburu et al. disclose the selective inhibition of NFAT activation by a peptide spanning the calcineurin targeting site of NFAT. While Aramburu et al. may discuss various aspects of

calcineurin binding, Applicant respectfully submits that Aramburu et al. **do not disclose the formation of the calcineurin/SOD complex**, as required by the claims of the present inventive subject matter. This deficiency is found in all of the references that the Examiner uses in this rejection (as is discussed above); therefore, Applicant respectfully submits that any combination of references, so combined in an attempt to achieve the presently claimed subject matter, would also be deficient in that the limitation regarding formation of the calcineurin/SOD complex would not be found in such a combination. Therefore, Applicant has shown that the Examiner has failed to prove a *prima facie* case of obviousness with respect to the claims.

Accordingly, Applicant respectfully submits that the claims are **not** obvious over the prior art of record in this case, and respectfully requests reconsideration and withdrawal of all outstanding rejections under 35 U.S.C. §103(a).

#### CONCLUSION

In view of the foregoing, applicant respectfully requests the Examiner to reconsider and withdraw the rejection of the claims and to allow all of the claims pending in this application.

If the Examiner has any questions or wishes to discuss this matter, the Examiner is welcomed to telephone the undersigned attorney.

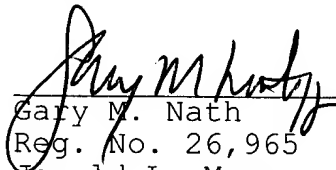
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Respectfully submitted,

**NATH & ASSOCIATES PLLC**

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